REMARKS

Claim amendments:

Claims 1, 28, 60 and 62 were amended.

Claim 1 was amended by adding 100uL to the preamble, replacing "comprising" with "consisting of" and adding "wherein the analysis does not involve an extraction step comprising evaporation and redissolving the extracted thyroid hormone, and the analysis is done in less than an hour".

Claim 28 was amended by adding 700uL to the preamble, replacing "comprising" with "consisting of" and adding "wherein the analysis does not involve an extraction step comprising evaporation and redissolving the extracted thyroid hormone, and the analysis is done in less than an hour".

Claim 60 was amended by adding "wherein the analysis does not involve an extraction step comprising evaporation and redissolving the extracted thyroid hormone, and the analysis is done in less than an hour".

Claim 62 was amended by adding 100uL to the preamble, adding "wherein the use consists of the steps of", and adding "wherein the analysis does not involve an extraction step comprising evaporation and redissolving the extracted thyroid hormone, and the analysis is done in less than an hour".

Claim rejections under 35 USC §103

As stated by the Examiner, the factual inquiries set forth in Graham v. John Deere Co., 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.
2. Ascertaining the differences between the prior art and the claims at issue.
3. Resolving the level of ordinary skill in the pertinent art.

4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

De Brabandere et al.

The Examiner objected to claims 1-8, 12-27, 58-59 and 60-65 as being unpatentable over De Brabandere et al.

The Examiner states that De Brabandere et al. discloses a method for the determination of thyroxine in serum using an internal standard and sample pretreatment consisting of protein precipitation and a two step liquid/liquid extraction procedure. The Examiner goes on to state that upon preparation of the standard solutions, approximately 3 mg of the thyroxine was used and dissolved in 10 mL of methanol with a few drops of HCl. The Examiner states that during extraction of thyroxine from the serum sample an exact serum volume, corresponding to approximately 50 ng of thyroxine was pipetted into a conical 5 mL vial. To the vial was added 50 ng of the internal standard and extraction was performed by allowing the mixture to equilibrate and 2 mL portion of acetone/30% HCl solution was added and mixed to deproteinize the sample. The Examiner states that the mixture was centrifuged and cooled and then centrifuged and cooled again in a refrigerator. The Examiner further states that the pH was adjusted with HCl. Finally, the Examiner states that De Brabandere et al. does not specifically disclose the sample volume to be tested, but it would have been obvious to one having ordinary skill in the art to modify the sample size so that it is 100 uL to analyze an extremely small sample without having to use a larger sample. The Examiner further states that it has been held that where the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum value by routine experimentation. In *re Aller, 220 F. 2d 454, 456, 105, USPQ 233, 235 (CCPA 1955).*

1. Determining the scope and contents of the prior art

As pointed out by the Examiner, the preparation of the sample taught in De Brabandere et al. is complex. The preparation time is estimated at between one and two and a half

hours. De Brabandere et al.'s method is a *reference method* and is meant to be used to harmonize routine methods. The first few lines of De Brabandere et al. state: "In clinical chemistry, reference methods are the key to an accuracy based harmonization of routine methods. They are applied to certification of reference materials for determination of target values in external and internal quality control materials and to the evaluation of routine methods on patient samples." Accordingly, the method disclosed by De Brabandere et al. is not meant to be used in the routine testing of patient samples. This may explain why the sample preparation is complex and lengthy.

The first step in De Brabandere et al.'s method is an extraction step which includes evaporation under a stream of nitrogen after which the residue is re-dissolved. The extraction step alone is calculated to take one to two hours.

Further, although De Brabandere et al. avoid stating how much serum they used, it appears to be somewhere between 0.6 mL and 1.5 mL. Because the motive behind De Brabandere et al. is to produce a *reference* method, there would be no incentive to reduce the sample size or to reduce the time needed to perform the analysis. The reference method is considered the gold standard, and not necessarily a method for routine testing.

In summary, De Brabandere et al.'s method (i) takes approximately 1 to 2 and a half hours, (ii) includes an extraction step, and (iii) requires between 0.6 mL and 1.5mL of serum per sample.

2. Ascertaining the differences between the prior art and the claims at issue

In contrast, the method of the present invention was developed specifically to test small samples, for example from newborns or children. See, for example, paragraphs [00027], [00033], [00080], [00097], [00101] and [00111]. Prior to the present method, small samples could not be used because the methods available for thyroid analysis all required large sample sizes.

Further, the present method does not include a separate extraction step outside of the on line extraction by liquid chromatography, as is required in De Brabandere et al. This is discussed at paragraph [0105] of the present invention which states:

> This example describes an isotope dilution tandem mass spectrometry method for the simultaneous determination of T4 and T3 in serum. The method is accurate, specific, precise (% CVs between 3.5 and 9) simple – requiring no extraction and only protein precipitation and fast (<7 min).

As stated above, and as pointed out by the Examiner, the preparation of the sample taught in De Brabandere et al. is complex. De Brabandere et al.'s method includes an extraction step, which is not present in the present invention. This is explained in paragraph [00019], at the last two sentences which state:

> Recently some reports of quantitative measurement of T4 and T3 by high performance liquid chromatography (HPLC), gas chromatography mass spectrometry (GC-MS), liquid chromatography mass spectrometry (LC-MS) or tandem mass spectrometry (LC-MS/MS) were published [16-20]. All those methods required extraction, derivatization and even prior chromatographic separation that are very time-consuming [21], [22].

Reference [20] is De Brabandere et al. The extraction step in De Brabandere et al. includes evaporation under a stream of nitrogen after which the residue was re-dissolved. The method of the present invention does not include any extraction step, other than the on line extraction during liquid chromatography, and is therefore inventive over De Brabandere et al.

Applicant amended claims 1, 28 and 62 by replacing "comprising" with "consisting of". To avoid any doubt, Applicant further added the phrase, "wherein the analysis does not involve an extraction step comprising evaporation and redissolving the extracted thyroid hormone, and the analysis is done in less than an hour".

Finally, the time required to perform the analysis in the present invention is as little as 6 minutes. De Brabandere et al.'s method of analysis is complicated and lengthy. The preparation time is estimated at between one and two and a half hours. In contrast, the present method is not complex and can be done in as little as 6 minutes (see paragraphs [00023], [00032], and [00097]).

Accordingly, the differences between the prior art and the present invention are threefold: (i) sample size, (ii) less complex sample preparation, (iii) and less time to perform the analysis. In essence, the state of the art teaches away from (i) using small sample size, (ii) using a simple method which does not include an extraction step and (iii) using less than one hour to analyze the sample. The state of the art is directed to a reference method of thyroid hormone analysis used to harmonize other methods. The reference method requires a large sample size, a complex preparation and is time consuming.

Applicant amended claims 1, 28 and 62 by inserting "100 uL", "700uL" and "100uL" in the preamble, respectively. The novel method of the present invention is not merely and optimization of an existing method, but a new and unobvious method developed to answer an unmet need. Small sample sizes may be analyzed from newborns and children, or in forensics.

Applicant amended claims, 1, 28 and 62 by inserting the phrase "wherein the analysis does not involve an extraction step comprising evaporation and redissolving the extracted thyroid hormone, and the analysis is done in less than an hour".

3. Resolving the level of ordinary skill in the pertinent art

Applicant submits the ordinary worker having regard to the available methods would not consider it possible to analyze thyroid hormones from a 100 uL sample because all of the methods in the art utilized a much larger sample size. There is no teaching or suggestion to use small sample sizes in the cited art. Further, the skilled worker may consider that a larger sample size is *required* because the methods available to him/her required extensive steps, including an extraction step. The skilled worker would also consider that extensive steps are required, because there is no motivation or teaching in the cited

references otherwise. Finally, the skilled worker would consider it necessary that the analysis take more than an hour, because the cited references to not teach or suggest otherwise.

4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

The present invention includes evidence of nonobviousness in that it teaches a novel method of thyroid hormone analysis from a small sample size. The present invention is not merely a discovery of the optimum value of the sample size using a known methodology as suggested by the Examiner, but a new method invented to answer an unmet need. The previous state of the art did not allow for the analysis of thyroid hormones from small sample sizes, for example from newborns or small children. Additional evidence of nonobviousness is that the present invention teaches a simple method of thyroid analysis, unlike the cited art which teach complex methods comprising extractions of thyroid hormones from the sample by evaporation under a stream of nitrogen after which the residue is re-dissolved. Finally, the present invention teaches that the analysis can be done in as little as 6 minutes – whereas the cited reference teaches that the analysis takes between 1 and 2 and a half hours. Any one of these three differences would make the invention non-obvious over the cited art. Including all three differences in the claims leaves no doubt that the present invention is not obvious over the cited art.

With regard to claims 4-5 and 7-8 in particular, the Examiner states that it would have been obvious to extract thyroxine from another biological sample other than serum. However, Applicant submits that as stated above, De Brabandere et al. does not render the amended independent claims obvious, and therefore claims 4-5 and 7-8 are also not obvious.

Regarding claims 60-62, the Examiner states that it would be obvious to assemble the reagents into a kit. However, Applicant submits that as stated above, De Brabandere et al. does not render the present invention obvious, and therefore claims 60-62 are also not obvious.

Regarding claims 64-65, the Examiner states that it would be obvious to use either API2000 or API3000. However, Applicant submits that as stated above, De Brabandere et al. does not render the amended independent claims obvious, and therefore claims 64-65 are also not obvious.

By these arguments and amendments, Applicant submits that the rejection is overcome.

De Brabandere et al. and Andrews et al.

The Examiner states that claims 10-11 are obvious with regard to De Brabandere et al. in view of Andrews et al. The Examiner states that Andrews et al. discloses a method to extract thyroxine from serum using acetonitrile to deproteinate the thyroxine sample.

Applicant submits that De Brabandere et al. did not use an approximately 100uL sample as required in the independent claims directed to thyroid hormone analysis from which claims 10-11 depend. As discussed above, De Brabandere et al. used a complex extraction procedure that likely required a larger sample size. Andrews et al. teaches the synthesis of thyroid analogs for radioimmunoassays (RIA). There is no teaching in Andrews et al. regarding the volume of sample required for analysis by mass spectrometry. Therefore it would not be obvious to a skilled worker to come to the invention of claims 10-11 with regard to De Brabandere et al. in view of Andrews et al.

DeBrabandere et al. and Draisci et al.

The Examiner states that claims 28-31, 34, 41-45, 47-53 and 55-57 are obvious with regard to DeBrabandere et al. in view of Draisci et al. because Draisci et al. discloses the analysis of steroid hormones.

Draisci et al discloses the analysis of steroid hormones using a complex sample preparation and extraction procedure. As disclosed in the "Sample preparation procedure" section of the paper, Draisci et al. uses a 2 mL sample of serum and urine. The sample is buffered and sonicated. Then the sample is purified by solid-phase extraction. The analytes are then eluted and the solvent is removed under a nitrogen stream and the residue is dissolved in 100 µL of methanol. The sample is then injected

into the LC-MS-MS system. Accordingly, the sample preparation method disclosed by Draisci et al. is complex and requires a much larger sample size than 700uL. Neither DeBrabandere et al. nor Draisci et al. disclose a simple sample preparation method using a small sample size. Claims 28-31, 34, 41-45, 47-53 and 55-57 cannot be obvious in view of DeBrabandere et al. and Draisci et al.

DeBrabandere et al. and Draisci et al.

The Examiner states that claims 32-33 and 35-36 are obvious for the reasons set out for claim 31 above.

Claims 32-33 and 35-36 are dependent on claim 31, which is further dependent on claim 28. As argued above, claims 28 and 31 are not obvious and therefore claims 32-33 and 35-36 cannot be obvious.

DeBrabandere et al., Draisci et al. and Andrews et al.

The Examiner states that claims 39-40 are obvious in view of DeBrabandere et al., Draisci et al., and Andrews et al. because Andrews et al. discloses a method of extracting thyroxine in which acetonitrile is used to deproteinate the sample.

Andrews et al. teaches the synthesis of thyroid analogs for radioimmunoassays (RIA). There is no teaching in Andrews et al. regarding the volume of sample required for analysis by mass spectrometry. Claims 39-40 are not obvious because the claim from which they depend (claim 28) is not obvious as argued above.

DeBrabandere et al., Draisci et al., and Jonsson et al.

The Examiner objected to claims 39-40 and 64-65 citing DeBrabandere et al., Andrews et al., and Jonsson et al. Jonsson et al. discloses a method and system for the determination of cortisol in saliva using acetonitrile to deproteinate the sample and teaches analysis using API 3000.

As discussed above, DeBrabandere et al. and Draisci et al. do not render the independent claims obvious. Jonsson et al. discloses the analysis of one steroid,

cortisol, from saliva samples. Jonsson et al. does not teach the analysis of thyroid hormones. Jonsson et al. uses a saliva sample size of 250 µL. The proteins in the sample were precipitated with acetonitrile. The samples were then mixed and conditioned at room temperature for 10 minutes after which the samples were centrifuged at 2700g for 15 minutes. The supernatants were evaporated in a nitrogen flow and dissolved in 100 µL methanol containing 0.5% acetic acid. Accordingly, Jonsson et al. teaches a complex sample preparation method including an evaporation step followed by reconstitution. Accordingly, Jonsson does not remedy the deficiencies of DeBrabandere et al. and Draisci et al.

Regarding claims 64-65, as discussed above, DeBrabandere et al. and Draisci et al. do not render the independent claims obvious and therefore the dependent claims can not be obvious.

Applicant requests that the rejection be withdrawn.

CONCLUSION

Applicant believes that it has fully responded to the Examiner's concerns, and that the claims are in condition for immediate allowance.

Please charge any deficiency or credit any overpayment in any fee required for this response, including any petition fee, to Deposit Account No. 502651.

In the event that any issues remain, the Examiner is invited to telephone the undersigned at (416) 865-7387 with any proposal to advance prosecution.

Yours very truly,

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